THE EFFECT OF CYCLIC TRITERPENES ON CHOLESTEROL SYNTHESIS FROM MEVALONATE IN RAT LIVER HOMOGENATES†

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Summary:

The cyclic triterpenes lanosterol, 24-methylenecycloartanol, euphol and α-amyrin have been shown to partially inhibit the incorporation of DL-mevalonate-2-^{14}C into the total non-saponifiable lipid and the digitonin-precipitable material of rat liver homogenates.

Introduction:

Inhibition of cholesterol synthesis from acetate has been shown both *in vitro* and *in vivo* by acyclic semi-, mono-, sesqui-, and triterpenes (Isler *et al.*, 1959). A "feedback" inhibition due to an effect on hydroxymethylglutaryl CoA reductase has been demonstrated in cholesterol-fed rats. (Siperstein and Guest, 1959; Bucher *et al.*, 1960; Siperstein and Fagan, 1966). A partial inhibition in the conversion of mevalonate to cholesterol in liver preparations of cholesterol-fed animals was also observed in several studies (Siperstein and Guest, 1959; Gould 1959; Bucher *et al.*, 1959). The sites of these post-MVA inhibitions were recently shown (Gould and Swyryd, 1966) to occur between

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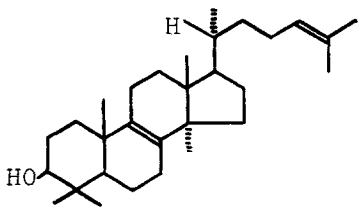
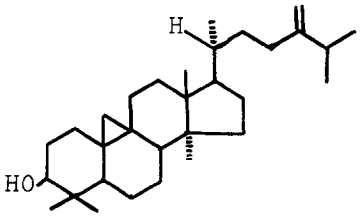
mevalonate and farnesyl pyrophosphate and after farnesyl pyrophosphate, probably between farnesyl pyrophosphate and squalene.

Cholesterol metabolites, the steroid hormones testosterone and estradiol (Carroll and Pritham, 1966) and the bile acids (Harris et al., 1967) also inhibit in vitro cholesterol synthesis in rat liver.

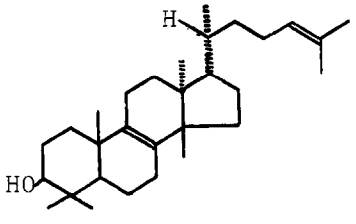
Due to the close structural and stereochemical similarity of some triterpenes to lanosterol (Fig. 1), an intermediate in cholesterol biosynthesis, and the fact that they may be regarded as



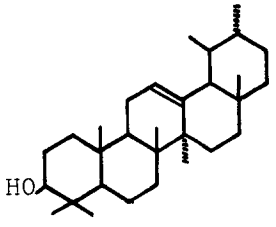
LANOSTEROL



24-METHYLENECYCLOARTANOL



EUPHOL



α-AMYRIN

Fig. 1

"end products" of the isoprenoid pathway, we undertook a preliminary investigation of the effect of some representative triterpenes on in vitro cholesterol synthesis.

Materials and Methods:

Lanosterol was a product of Mann Research Laboratories and contained 40% (w/w) dihydrolanosterol as determined by gas chromatography

on an SE30 column. Though a purified sample of lanosterol was prepared the inhibition studies revealed no significant difference between it and the commercial sample. α-Amyrin was obtained from Koch-Light Laboratories and showed one peak on the gas chromatograph. Euphol was a gift from Dr. Vilkas and, likewise, showed only one peak. The 24-methylenecycloartanol contained approximately 10% (w/w) cycloartenol. No attempt was made to purify it further. It was a gift from Dr. Heftmann.

Rat liver homogenates were prepared by the method of Bucher and McGarrahan (1956) using 2.5 ml of buffer for each gram of liver. The incubation mixtures were as described in the Tables. The triterpenes were dispersed in 2% gelatin solution or propylene glycol by sonication.

The non-saponifiable material was isolated as an ether extract and aliquots taken for counting. Digitonides were precipitated from aliquots of the non-saponifiable material by the method of Holmes and Benz (1960) and the 3β-hydroxysterols liberated for counting by the addition of pyridine according to the procedure of Avoy et al. (1965). For the isolation of the polyprenols the combined saponified solutions for each terpenoid concentration were hydrolysed with acid according to the method of Popjak (1959). Radioactivity was measured in a liquid scintillation spectrophotometer.

Results and Discussion:

In Table 1 is shown the distribution of radioactivity from mevalonic acid-2-^{14}C into the non-saponifiable, the digitonin-precipitable and the polyprenol fractions for incubations of rat liver homogenates with terpenoids dispersed in 2% gelatin. It is clear that the reduced incorporations of radioactivity into the digitonin precipitable fraction in the treated runs can be attributed in large part to the reduced incorporation into the corresponding

Table 1: Distribution of radioactivity from mevalonate-2-^{14}C into the non-saponifiable lipid (NS), digitonin precipitable fraction (DPF) and the polyprenol fraction (PF) of rat liver homogenate in the presence of triterpenes dispersed in 2% gelatin.

Triterpene	NS	DPF	PF
Control	100 ± 0	100 ± 2	2.4
	(31,200)	(16,000)	(4,300)
Lanosterol (0.5 mg)	60 ± 13	63 ± 18	5.2
Lanosterol (1.0 mg)	48 ± 6	44 ± 7	5.5
Lanosterol (2.0 mg)	38 ± 1	44 ± 1	8.3
α-Amyrin (1.0 mg)	83 ± 2	51 ± 4	---
24-Methylenecycloartanol (1.0 mg)	94 ± 3	79 ± 9	---
Euphol (1.0 mg)	62 ± 11	64 ± 18	7.2
Cholesterol (1.0 mg)	63 ± 7	68 ± 9	5.5

Each reaction mixture contained 1 ml of the 500 x g supernatant of the homogenate obtained from the combined livers of two male rats (200-300g); 2.0μmoles NADP; 3.5μmoles ATP; 0.5μmoles/0.08μC (177, 600 dpm) DL-mevalonate-2-^{14}C, and the terpenoid suspension (0.15 ml). The controls contained 0.15 ml of 2% gelatin. The mixtures were made up to 3 ml with homogenizing medium then incubated at 37° for 1 hour. The results in the NS and DPF columns are expressed as percentages of the control. The results in the PF column are percentage incorporations from mevalonic acid. The values in parentheses represent radioactivity in dpm in the control fractions. The error data represent percent deviations from the mean. All experiments were conducted at least in duplicate

non-saponifiable material. The accumulation of radioactivity in the polyprenol fractions in those cases were significant inhibition into the non-saponifiable fraction occurred, indicates that the site(s) of inhibition probably lies between the formation of isopentenyl pyrophosphate and the dimerization of farnesyl pyrophosphate. The almost insignificant effect of 24-methylenecycloartanol in this system may be due to the very poor dispersion obtained in the gelatin solution. Differences in the degree of inhibition occur from run to run with all the triterpenes. It is probable that the difficulty of obtaining optimal contact between

the extremely water-insoluble triterpenes and the enzymes involved account for this. In Table 2 is shown the data for 24-methylene-cycloartanol and euphol in propyleneglycol in which both compounds

Table 2: Distribution of radioactivity from mevalonate-2-^{14}C into the non-saponifiable lipid (NS) and the digitonin precipitable fraction (DPF) of rat liver homogenate in the presence of triterpenes dispersed in propyleneglycol.

Triterpene	NS	DPF
Control	100 ± 5	100 ± 9
	(38,500)	(11,900)
24-Methylene-cycloartanol (0.5 mg)	69 ± 13	72 ± 3
" (1.0 mg)	65 ± 11	64 ± 6
Euphol (1.0 mg)	77 ± 5	46 ± 5

The conditions of incubation and the presentation of data are as described in Table 1. The controls contained 0.15 mg of propyleneglycol.

are better dispersed. The data again show reduced incorporation into the non-saponifiable fraction. In this system however, euphol appears to have exerted an additional inhibitory effect at a stage subsequent to the dimerization of farnesyl pyrophosphate (c.f. α-amyrin in Table 1).

It is clear that the triterpenes are not acting by a simple dilution effect at the level of lanosterol for if such were the case the incorporation of radioactivity into the non-saponifiable fraction would remain unaltered. It may be argued that lanosterol acts via its transformation to cholesterol. However, such a transformation is not likely for the other triterpenes. The accumulation of the polyprenols and the TPNH requirement for the dimerization of farnesyl pyrophosphate might suggest an effect on TPNH formation under the conditions described in Table 1.

In view of the influence on *in vitro* cholesterol synthesis by the C_{8-16} fatty acids (Fluck and Pritham, 1961); two representative

sex hormones; several acyclic terpenes and, in the present work some cyclic triterpenes, there may be a relatively non-specific lipid effect operating on this pathway.

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